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Crystal Structure of the Disabled-1 PTB domain-ApoER2 Peptide Complex

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Beamline(s): X12C

Introduction: The formation of the mammalian six-layered neocortex depends on a signaling pathway that involves the large signaling protein Reelin, the very-low-density lipoprotein receptor (VLDLR), the Apolipoprotein E receptor-2 (ApoER2), and the adaptor protein Disabled-1 (Dab1) [1-4]. Dab1 binds to a sequence containing the consensus 'NPxY' motif in the cytoplasmic domains of both receptors through its N-terminal phosphotyrosine binding (PTB) domain [4,5]. The Dab1 PTB domain has also been shown to bind to PI-4,5P_2 through a site that does not compete with peptide binding, suggesting that the activity of Dab1 may also depend on or be regulated by recruitment to phosphoinositide-enriched membrane compartments [6]. To understand the basis for selective recognition of peptide with an unphosphorylated tyrosine in the NPxY motif, and to identify potential sites for PI binding, we determined the crystal structure of the Dab1 PTB domain in complex with a 14-residue peptide from the ApoER2 cytoplasmic tail.

Methods and Materials: Crystals of the Dab1-peptide complex were grown using vapor diffusion methods and were cryogenically frozen for transport to the National Synchrotron Light Source (Brookhaven National Laboratory). All diffraction data were collected at 100 K in a cryo-stream. Data collected on a crystal of selenomethionine-derivatized Dab1 protein containing a L87M mutation were used for MAD phasing. Three wavelengths of data were collected at the Se absorption edge, peak, and high energy remote to a resolution of 2.0 angstroms. These data and a 1.5 angstrom native data set collected earlier at the Cornell High Energy Synchrotron Source (Cornell University) were used to calculate the electron density map and build the model.

Results: The Dab1 PTB domain exhibits a canonical PTB fold, in which seven central beta-strands form two anti-parallel, near-orthogonal beta-sheets, capped by a long C-terminal alpha-helix (Fig. 1). The structure clarifies the basis for sequence selectivity of the PTB domain in peptide binding, including the unusual preference of Dab1 for an unphosphorylated tyrosine within the NPxY motif. Selection against phosphotyrosine by Dab1 is established by a cleft created by the short loops connecting beta-strand 4 with strand 5 and strand 6 with strand 7, which closely approach the tyrosine side chain and should sterically exclude the bulkier phosphotyrosine (Fig. 1). In addition, the structure reveals that a conserved group of basic residues within the PTB domain come together on the face opposite the bound peptide to create a region of positive electrostatic surface potential that is proposed to delimit the PI-4,5P_2 binding site (Fig. 2).

Conclusions: Although it is not known whether the NPxY motif of either lipoprotein receptor tail undergoes phosphorylation, the preference of the Dab1 PTB domain for binding a sequence with unphosphorylated tyrosine shows it would be possible to regulate downstream signaling by modulating the tyrosine phosphorylation state of the receptor tails. In addition, these findings suggest that membrane recruitment of the Dab1 PTB domain to PI-4,5P_2-rich patches in the plasma membrane facilitates binding of the cytoplasmic domains of the Reelin receptors. Control of membrane localization by PI-binding may regulate Dab1 activity by facilitating downstream events that accompany peptide binding, such as tyrosine phosphorylation by membrane-associated kinases. In addition, the ability of Dab1 to bind to PIs may itself be diminished when the tyrosine residues adjacent to the PTB domain are phosphorylated upon activation of a Reelin signal.

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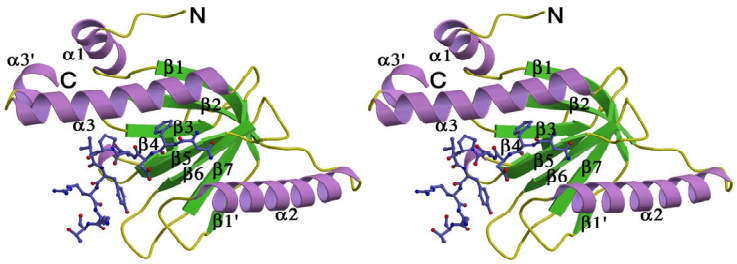

Fig. 1 Ribbon representation of the Dab1 PTB domain in stereo colored by secondary structure, with bound peptide rendered in ball and stick form.

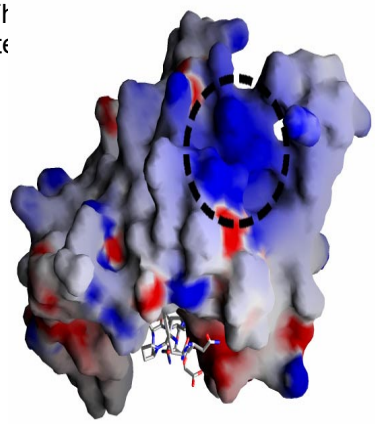

Fig. 2 A molecular surface representation of the Dab1 PTB domain shaded by electrostatic potential (-15 kt/e, red to +15 kt/e, blue) and oriented with the peptide-binding groove at the base and the N-terminal residues of the bound peptide projecting towards the viewer. The distinct region of electrostatic potential is indicated with a dotted circle.